Dpr 282

California Department of Food and Agriculture
Center for Analytical Chemistry
Environmental Monitoring Section
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Title: Determination of MITC in Air By GC/NPD or GC/TSD

1. Scope

This section method (SM) is for the analysis of MITC from air sample tubes using GC/NPD or GC/TSD and is to be followed by all authorized EMON section personnel. The reporting limit of MITC is 0.2 µg per sample by NPD and 0.05 µg per sample by TSD.

2. Principle:

Residues of MITC (methyl isothiocyanate), CH_3 –N=C=S, that has been absorbed from the air onto activated charcoal is desorbed from the charcoal with 0.1% CS_2 in ethyl acetate. It is analyzed by gas chromatography using a nitrogen phosphorus detector (GC/NPD or GC/TSD).

3. Safety:

3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.
3.2 All solvents should be handled with care in a ventilated area.

4. Interferences:

No known matrix interferences that cause quantitative problems above the established reporting level. However, nitrogen or phosphorus compounds with the same retention time may interfere with the quantification.

5. Apparatus and Equipment:

5.1 Test tubes, 25 mL, with Teflon lined screw cap
5.2 Assorted pipettes and micro syringes
5.3 Volumetric flasks
5.4 Files able to score the sample tubes or a Dremel (an electric rotary flex shaft tool) with ¾" diamond saw
5.5 Thermolyne Vortex Maxi mixer
5.6 Forceps
5.7 Plug puller, Supelco #2-0596

5.8 HP 6890 gas chromatograph with NPD or Varian 3800 gas chromatography with TSD.

6. Reagents and Supplies

6.1 Carbon disulfide, nanograde
6.2 Standards:
Obtain 1.0 mg/mL reference standards of MITC from the Standard Repository, CAC, CDFA, 3292 Meadowview Road, Ca 95832.
MITC CAS Number 556-61-6
6.3 Charcoal tubes: SKC #226-09, lot #120
6.4 Ethyl Acetate, pesticide residue grade
6.5 Filters, Nylon Acrodisc, 0.45 μm, Gelman Sciences

7. Standards Preparation:

7.1 Dilute the 1.0 mg/mL standards, obtained from the CDFA/CAC Standards Repository, with the solution of 0.1% CS_2 in ethyl acetate. The working standards shall be prepared to cover the linear range from 0.025ηg/μL to 5.0 ηg/μL. The levels we prepared are 0.025, 0.05, 0.10, 0.25, 0.50, 1.0, 5.0 ηg/μL

7.2 Keep all standards in designated refrigerator for storage.

7.3 The expiration date of each mixed working standard is six months from the preparation date.

8. Sample Preservation and Storage:

All samples to be extracted shall be stored in a designated freezer and all sample extracts shall be stored in a designated refrigerator (0-5 °C).

9. Test Sample Preparation:

9.1 Sample Preparation

9.1.1 Remove samples from refrigerator to the laboratory bench and allow the samples to warm to near ambient temperature.
9.1.2 Fold a white sheet of 8x11 printer paper into quarters, reopen and place it under the tube to catch any spilled charcoal.

9.1.3 Score the tube with a file or a dremel near the wide end of the metal spring. Break the tube by holding it with both hands at each side of the cut, having the cut pointing away from you and push the tube by the tips of your thumbs.

9.1.4 Use a 9" disposable pipette to push all tube material into a test tube containing 5.0 mL of 0.1% CS_2 in ethyl acetate and cap the tube immediately.

9.1.5 Allow samples to desorb for 30 minutes and vortex them occasionally

9.1.6 Filter the mixture through a nylon Acrodisc and collect it in two auto-sampler vials. Cap them immediately. Store one vial in a designated freezer for possible later use

9.2 Spike extraction: Break both ends of a charcoal tube with a file or a dremel. Use a syringe to spike a known amount of MITC through glass wool onto the center of charcoal section. Follow the steps 9.1.3 through 9.1.6 to do extraction.

9.3 Confirmation by mass spectrometer may be performed on GC/MSD on Sim mode (ions 73, 58, 45), if required.

10 Instrument Calibration:

10.1 The concentrations of the standards we used for establishing the calibration curve were 0.025, 0.05, 0.10, 0.25, 0.50, 1.0 and 5.0ng/μL

10.2 A solution of 0.04 ng/μL equals to the RL of 0.2 μg/sample.

11 Analysis:

11.1 Injection Scheme

Follow the sequence of a set of calibration standards, a matrix blank, a matrix spike, a set of 12 or less test samples, a set of standards, etc.

11.2 Instrumentations and operating conditions:

11.2.1 Varian gas chromatograph model 3800 with dual injectors and dual TSD detectors. Varian auto sampler model CP8400. Varian software Galaxie version

Column: DB-FFAP, 10 m x 0.53 mm x 1.0 μm

Gas Flow:
Carrier gas, constant flow (Helium) at 10 mL/min
He makeup flow, 25 mL/min
Hydrogen flow, 5.4 mL/min
Air flow, 190 mL/min.

Temperature:
Oven temperature program,

Rate(°C/min)	Temperature (°C)	Time (minute)
Initial	45	1
15	90	4
50	220	6.6

Injector temperature: 200 °C
Detector temperature: 250 °C

Retention time: 3.0 min

Injection Volume: 2.0 μL

11.2.2 HP 6890 gas chromatograph with dual injectors and dual NPD detectors.
Column: DB-FFAP, 10 m x 0.53 mm x 1.0 μm

Gas Flow:
Carrier gas, constant flow (Helium) at 9 mL/min
He makeup + carrier flow, 12 mL/min
Hydrogen flow, 3.0 mL/min
Air flow, 60 mL/min.

Temperature:
Oven temperature program,
Initial temp.: 45°C for 7 minutes
Rate: 40°C/minute
Final temp.: 200°C
Injector temperature: 220 °C
Detector temperature: 250 °C

Retention time: 5.8 min

Injection Volume: 2.0 µL

11.2.3 Mass spectrometer and Operating Parameters

Model: Agilent 6890 equipped with 5972 mass selective detector
Column: RX-200 trifluoropropylmethyl polysiloxane, 60m x 0.32 mm x 1.5 µm cat #15072

Injector temperature:	210 °C
Initial column temp:	40 °C for 4 minutes
Ramp 1 rate:	12 °C per minutes
Final temperature 1:	160 °C
Ramp 2 rate:	40 °C per minutes
Final temperature 2:	240 °C for 1 minute

Mass spectrometer parameters:

Transfer line heater:	280 °C
Dwell time:	30 milliseconds
Selected ions:	72, 73, 45, 58
Injection volume:	2.0 µL
Retention time:	12.01 minutes

12. Quality Control:

12.1 A six point standard curve of 0.025, 0.05, 0.1, 0.5, 1.00 and 5.00 µg/mL shall be obtained at the beginning and the end of each set of samples for calculating the response factors and checking the instrument performance.

12.2 Each set of samples shall have a matrix blank and minimum of one matrix spike sample. Each set contains up to 10 samples.

12.3 The matrix blank shall be free of target compounds.

12.4 The recoveries of the matrix spike shall be within the control limits.

12.5 The retention time shall be within ± 5 seconds of that of the standard.

12.6 The sample must be diluted if results fall outside the linear range of the standard curve.

12.7 Bracketing standard response shall have a percent change less than 10 %

12.8 Method Detection Limits (MDL)
The method detection limit refers to the lowest concentration of analyte that a method can detect reliably. To determine the MDL, 7 replicate charcoal tubes samples are spiked with 0.10μg of MITC solution. The standard deviation of the findings from the spiked sample are used to calculate the MDL using the follow equation:

MDL = tS

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicate used to determine the MDL, t=3.143.

12.9 Reporting limit (RL):
The reporting limit (RL) refers to the level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. Per client agreement, the RL is chosen in a range 1-5 times the MDL., unless otherwise agreed upon by client.

MDL data and the RL are tabulated in Appendix 1

12.10 Method Validation Recovery Data and Control Limits:

12.10.1 The method validation consisted of five sample sets. Each set included three levels of fortification (0.4, 3.0 and 8.0 μg/sample) and a method blank. A reagent blank shall be included when a new lot of solvent is used for extraction. All spikes, method blank and reagent blank samples were processed through the entire analytical method.

12.10.2 Upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the average % recovery, respectively.

Method validation results and control limits are tabulated in Appendix 2

13. Calculations:

13.1 The quantification is based on the area counts of the target compound. The calculation is based on external standard (ESTD) and linear fit

13.2 The correlation coefficient, slope, intercept of the linear regression line are calculated once the calibration standards are defined. The equation for calculating analytes is as follows:

$$y = mx+b$$

Where: y = peak response
m = slope
b = intercept
x = concentration of compound

When the unit and the dilution factor are entered correctly in the analysis sequence, the software will then correctly generate the results.

13.3 Results can be manually calculated by a single point standard. The unit is µg per sample for all samples. This calculation is to verify the results derived from the instrument

The general equation is as follows:

$$\mu g = \frac{\text{(sample peak area) (std. conc. ng/µL) (std. vol. injected) (sample final vol., (mL))(1000 µL/mL)}}{\text{(std. peak area) (sample vol. injected) (1000ng/µg)}}$$

14.1 Acceptance Criteria:

14.1.1 Peak retention time between standards, QC spikes and unknowns shall be within 5 seconds. If there is a known reason of retention time shifting, an explanation memo shall be included.

14.1.2 Peak response shall be within the calibration range

14.1.3 The R^2 of calibration curve or overlay calibration curves shall be greater than 0.990.

14.1.4 Recoveries of spike QC shall be within the established control range, otherwise a rerun shall be performed.

14.1.5 The result by manual single point calculation shall agree with that by the Galaxie software.

14.2 Reporting:

14.2.1 Sample results are reported out according to the client's analytical laboratory specification sheet.
14.2.2 Fill out COC, QC sheet, and control chart.
14.2.3 Prepare data package. Peer review. Report.

15 Discussion

15.1 This method is a revision of the reference 16.2. One modification was to add the Varian's Thermionic Specific Detect (TSD) to the method. The response of the TSD is more sensitive and more stable than that of the NPD. Therefore, it allows us to improve the reporting limit from 0.2 μg/sample to 0.05 μg/sample.
15.2 The GC-MSD parameters have also been modified. A longer column with thicker film allows the column to retain MITC longer and to improve separation from interference peaks.

16. References:

16.1 ICI Americas Inc., "Methyl Isothiocyanate from Metham-Sodium Determination in Air" #RRC-35, August 26, 1982.
16.2 Center of Analytical Chemistry, California Department of Food and Agriculture, "MITC in Air Sample by GC/NPD" EMON#41.9, 10/28/99. A revision of 7/08/1993.

APPENDIX I

The Method Detection Limit (MDL) data from NPD

	MITC Spiked (μg)	MITC Found (μg)	%
MDL-spike1	0.15	0.1314	87.6%
MDL-spike2	0.15	0.1292	86.1%
MDL-spike3	0.15	0.1245	83.0%
MDL-spike4	0.15	0.1297	86.5%
MDL-spike5	0.15	0.1191	79.4%
MDL-spike6	0.15	0.1295	86.3%
MDL-spike7	0.15	0.1211	80.7%
Average		0.1264	84.2%
STDEV		0.0048	3.20%
MDL=3.143xSTDEV		0.0151	
RL*		0.20	

*Due to NPD response consistency problem, we set the RL at larger than 5 X of its MDL

The Method Detection Limit (MDL) data from TSD

	MITC Spiked (μg)	MITC Found by Detector 1 (μg)	MITC Found by Detector 2 (μg)
MDL-spike1	0.10	0.1085	0.0930
MDL-spike2	0.10	0.1100	0.0915
MDL-spike3	0.10	0.1190	0.1010
MDL-spike4	0.10	0.1210	0.1010
MDL-spike5	0.10	0.1200	0.1015
MDL-spike6	0.10	0.1165	0.1000
MDL-spike7	0.10	0.1155	0.1000
Average		0.1155	0.0983
STDEV		0.0049	0.0042
MDL=3.143xSTDEV		0.015	0.013
RL		0.05	0.05

Appendix 2
Method Validation Data for MITC from NPD

Spike Level MITC Spiked (µg)	Set1 MITC found (%)	Set 2 MITC found (%)	Set 3 MITC found (%)	Set 4 MITC found (%)	Set 5 MITC found (%)
0.4	83.8	91.2	89.0	104.3	86.4
3.0	82.4	88.4	96.0	102.8	87.9
8.0	84.7	85.7	77.0	80.8	80.6

Average		88.07%
Standard Deviation		7.817%
Upper warning Limit	2x Stdev	103.70%
Upper Control Limit	3x Stdev	111.52%
Lower warning Limit	2x Stdev	72.43%
Lower Control Limit	3x Stdev	64.61%

Method Validation Data for MITC from TSD

Spike Level MITC Spiked (µg)	Set1 MITC found (%)	Set 2 MITC found (%)	Set 3 MITC found (%)	Set 4 MITC found (%)	Set 5 MITC found (%)
0.1	102.0	99.0	96.0	99.0	97.0
0.4	82.5	87.5	87.5	90.0	85.0
3.0	97.3	106.7	103.3	93.0	112.7
8.0	97.8	98.5	92.3	86.9	100.3

Average		95.72%
Standard Deviation		7.67%
Upper warning Limit	2x Stdev	111.06.%
Upper Control Limit	3x Stdev	118.73%
Lower warning Limit	2x Stdev	80.37%
Lower Control Limit	3x Stdev	72.69%

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Revision Log:

Date	What was Revised? Why?
9/20/04	Formatting to ISO format
9/20/04	Adding a new instrument (Varian TSD) and the parameters to the method
9/20/04	Reducing reporting limit to 0.05 µg from 0.2 µg
9/20/04	Adding new validation data obtained from TSD to the method
9/20/04	Modifying the confirmation MSD method parameters